

samples: scr-c028 in stg.

MINIPREP FOR DNA PREPARATION

- 1) INOCULATE CLONES INTO 3.0 ML OF LB MEDIA. OVERNIGHT AT 37 DEG.
- 2) THE FOLLOWING DAY LABEL 1.5 ML. STERILIZED EPENDORF TUBES TO BE USED.
- 3) FILL 3/4 OF EACH TUBE WITH THE CULTURED BACTERIA. CLOSE THE CAP.

- 4) SPIN TUBES USING THE EPENDORF CENTRIFUGE FOR ONE MINUTE AT 6000 RPM.
- 5) PREPARE THE POT FOR BOILING WATER.
- 6) DECANIT THE SUPERNATANT, LEAVING A LITTLE BIT IN THE TUBE.
- 7) RESUSPEND THE PELLET BY VORTEXING.

- 8) ADD 120 MICRO ML OF STEL-1 SOLUTION. MIX GENTLY BY INVERTING THE TUBES. DO NOT VORTEX.
- 9) KEEP THE TUBES IN BOILING WATER FOR EXACTLY 1 MINUTE AND THEN IMMEDIATELY TRANSFER THE TUBES TO A BLOCK OF ICE.
- 10) KEEP THE TUBES IN ICE FOR 5 MINUTES.

- 11) SPIN THE TUBES IN THE MICROFUGE FOR 5 MINUTES AT THE HIGHEST SPEED.
- 12) USING A STERILIZED TOOTHPIK PICK UP THE PRECIPITATE AND DISCARD IT, LEAVING THE SUPERNATANT BEHIND.
- 13) ADD 120 MICRO ML OF 2-PROPRANOL (same quantity as the STEL-1 solution) 2-ISOPRANOL PRECIPITATES THE DNA. MIX THE TUBES BY INVERSION AND KEEP ON ICE FOR 5 MINUTES.

- 14) DECANT THE SUPERNATANT AND LEAVE THE TUBES TO AIR DRY IN AN INVERTED POSITION.
- 15) TO HASTEN THIS STEP, YOU MAY ADD 200 MICRO ML OF 100% ALCOHOL WITHOUT DISTURBING THE PELLET AND ALLOW TO AIR DRY AS BEFORE.
- 16) ONCE THE PELLET IS DRY, WHICH CAN BE MADE OUT BY THE POWDERY APPEARANCE OF THE PELLET, RESUSPEND IN 60 MICRO ML OF TE WITH RNASE.

WORKING SOLUTION OF TE WITH RNASE

2 MICRO ML OF RNASE (20 mg/ml) IN 1 ML OF TE
16) THESE DNA CAN BE FROZEN AT -20 TEMPERATURE IF REQUIRED

3 out
7 out
2 out

24000

24000

200

-51-

1) Vol I - 2001 in 3000.

$$1-12 \rightarrow 25\mu\text{L} + 3\mu\text{L } 10 \times 153 + 0.3\mu\text{L } 100 \times 155A = 1\mu\text{L Vol I}$$

$$\text{Vol I} \times 1/4 = 3.6\mu\text{L } 155A \text{ } 100 \times$$

$$42\mu\text{L } 10 \times 5$$

$$4\mu\text{L Vol I}$$

$$\text{Vol I} \rightarrow 1\mu\text{L} + 24\mu\text{L } 120 +$$

1 1/2 370C

→ 10% CO₂ if not had cut

2μL × 155A (1/4)

3μL 110

3μL 4-12

2μL × 155A (1/4)

↑
27μL
↑

2) 40μL BUI - 0.1g

→ 43μL 10 × 153

43μL 10 × 155A

1 μL BUI

85μL 120

$$\text{Vol I} \times 1/4 \rightarrow 18.2\mu\text{L } 10 \times 153$$

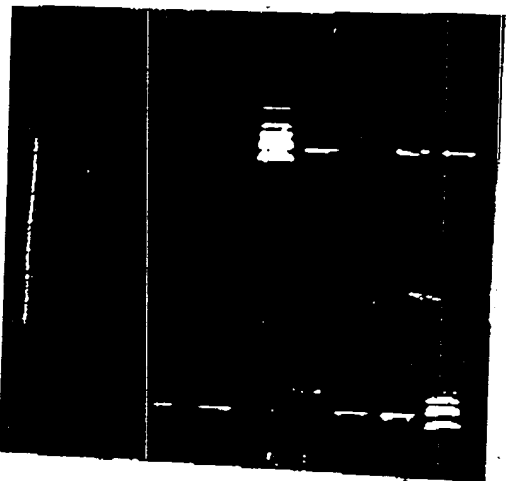
18.2μL 10 × 155A

14μL BUI

140μL 120

13μL Tube

→ 2 1/2 370C



200 - 701